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Hormonal priming alleviates salt stress in hot Pepper (*Capsicum annuum* L.)

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Abstract

Germination and seedling establishment are critical stages in the life cycle of plants especially under stress conditions. Different methodologies have been adopted by plant physiologists in different crops to alleviate salt stress. Seed priming has proven beneficial in this regard in many important agricultural crops. The effect of seed priming with salicylic acid (SA) and acetylsalicylic acid (ASA) in improving seed vigour and salt tolerance of hot pepper seedlings was evaluated. Concentrations over 1.0 mM of ASA or SA showed adverse effects on seed emergence. Seeds primed with SA (0.8 mM) and ASA (0.2 mM) were sown in medium at different salinity levels [0, 3, 6 and 9 dS m^{-1}]. Both, SA and ASA treatments showed significantly better results over the control by improvement in time taken to 50% emergence, final emergence percentage, root and shoot length, seedling fresh and dry weight and seedling vigour. Overall, acetylsalicylic acid exhibited superiority over salicylic acid. Our results indicate that hormonal priming, especially with acetylsalicylic acid, can be a good treatment for hot pepper to enhance uniformity of emergence and seedling establishment under normal as well as saline conditions.

Keywords: Seed priming, salicylic acid, acetylsalicylic acid, salinity tolerance, seedling vigour, hot pepper

Introduction

Abiotic stresses are the principle threat to plant growth and crop productivity all over the world. Salinity adversely affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. This is the most serious threat to agriculture and major environmental factor that limits crop growth and productivity (Ashraf *et al.,* 2008; Munns and Tester, 2008). Almost 20% of cultivated area of the world and half of the world's irrigated lands are stressed by the salinity (Chinnusamy *et al*., 2005). In Pakistan, almost 6.3 mha of land is affected by salinity, which is estimated to be 14% of irrigated land and this causes a yield loss of 64% (Afzal *et al*., 2005).

Hot pepper (*Capsicum annuum* L.) is an important vegetable as well as spice crop of the Pakistan. Hot peppes or Chillies are rich in antioxidants, vitamin C, pro-vitamin A, E, P (citrin), B_1 (thiamine), B_2 (riboflavin), and B_3 (niacin) (Bosland and Votava, 1999). Peppers are best known in pharmaceutical industry because of its capsaicin contents, which is used as a pain relieving medication. The hot pepper production is estimated to be 120.46 thousand tons from an area of 59.4 thousand hectares with average yield of 2.028 tons per hectare (Govt. of Pakistan, 2006). Chilli is a valuable crop as it earned Rs. 1.127 billion during 2003-04 (Anonymous, 2006). Moreover, the yield gap for chillies is too high; 1 to 2 tons per hectare in Pakistan as compared to 20 tons in other pepper producing countries like China. One of the main problems of this yield gap is high soil salinity in southern Punjab and Sindh; characteristics of arid climate.

Chilli is regarded as a sensitive (Haman, 2000) to moderately sensitive crop to salts (Kanber *et al*., 1992). Seed germination and early seedling growth are considered as the most sensitive stages to salinity stress (Ashraf and Foolad, 2005) in most of the crops. Germination and emergence of pepper seeds is also slow and non-uniform under normal as well as stress conditions (Demir and Okcu, 2004). Soil salinity, if not properly managed, can become a limiting factor for chilli stand establishment causing decrease in germination rate and germination percentage of pepper seeds. Moreover, chilli yield is reduced by 14% for every increase in unit of salinity above its threshold (Rhoades *et al*., 1992).

Seed priming is a technique of seed enhancements that improves germination or seedling growth. Seed priming enhances seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination in different crops (Cantliffe, 2003). It permits seedling development in a wide range of agro-climatic conditions and decreases sensitivity to external factors (Ashraf and Foolad, 2005; Welbaum, *et al*., 1998). Seeds performance of various crops can be improved by inclusion of plant growth regulators and hormones during priming and other pre-sowing treatments (Lee *et al.*, 1998). Hormone like salicylic acid has also proved for alleviating salinity stress in wheat (Hamada and Al-Hakimi, 2001; Afzal *et al*., 2005). Acetyl salicylic acid has proved

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to protect muskmelon seedlings against drought stress (Korkmaz *et al.*, 2007) and sweet pepper seeds against low temperature (Korkmaz, 2005). Similarly, acetyl salicylic acid (ASA) and salicylic acid (SA) have been showed to lessen the deleterious effects of abiotic stress on tomato and bean (Senaratna *et al.*, 2000).

Keeping in view the role of salicylic and acetyl salicylic acid in improving vigour under salt conditions and beneficial effects of seed priming in various crops, the present study was conducted to investigate the role of ASA and SA as priming agent in hot pepper under saline conditions.

Material and Methods

This study was conducted in the Vegetable Seed Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, to determine the effect of different pre-sowing seed treatments on emergence and seedling vigour of hot pepper (*Capsicum annuum* L.) cv. Hot Queen under saline conditions. The seeds of pepper cultivar Hot Queen were obtained from Yousuf Seed Corporation (Registered), Faisalabad, Pakistan. The initial seed moisture was 8 % (dry weight basis).

Priming treatment

On the basis of previous observations (unpublished data), best doses of both priming agents were selected for this experiment to observe the effects of seed priming on performance of hot pepper under different salinity levels. Seeds were surface sterilized by dipping in sodium hypochlorite (5 %) solution for 5 min and dried on filter paper. These surface sterilized seeds were primed in aerated solution of salicylic acid and acetylsalicylic acid at concentrations of 0.8 and 0.2 m*M,* respectively, for 48 h at 25 ± 2 ^{o}C under dark conditions. After respective priming treatment for specific period, seeds were washed with distilled water (Khan, 1992). The seeds were then dried at room temperature on filter paper in shade for 24 h (Bennett and Waters, 1987). Seeds were then packed in polythene bags and stored in a refrigerator at 5 ± 2 ^{o}C for further use.

Emergence test

Primed and un-primed seeds were sown in plastic trays filled with washed fine sand and irrigated with half-strength Hoagland solution containing different salt levels [0 (Control), 3, 6 and 9 dS m^{-1}], kept at 25 $\pm$ 2 ^{o}C under 16 h photoperiod in an incubator.

Data collection

Seed was considered as emerged when 3-4 cm long radicle was visible out of the soil surface. Data were recorded daily on emergence for 14 days following seedling evaluation protocol given in the handbook of the Association of Official Seed Analysis (1990). The time taken to 50% emergence [E_{50}] was calculated according to the formula of Coolbear *et al.* (1984), as modified by Farooq *et al.* (2005). Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981). Final emergence percentage (FEP) was determined at the end of experiment. Emergence index (EI) was calculated according to the procedure given in the handbook of the Association of Official Seed Analysis (1983).

Ten normal seedlings from each replication were taken at random at final count and shoot and root length was measured from collar region with the help of measuring tape after 14 days of sowing and averaged to obtain mean shoot and root length. Seedling fresh weight was obtained using ten normal seedlings from each replication after 14 days of sowing and dry weight was obtained by drying at 105 ^{o}C in oven for 24 h.

Statistical analysis

The experiment was arranged according to completely randomized design with four replicates, each replicate having 50 seeds. Data recorded were analyzed statistically using Fisher's analysis of variance technique and Duncan's Multiple Range Test at 5% probability level to compare the differences among treatment means (Steel *et al*., 1997).

Results

Results for FEP were found to be significant (P<0.05) for all treatments under saline conditions (Figure 1). Salinity reduced the final emergence percentage in all the treatments. However, FEP was higher for primed seeds than non-primed seeds at all the salinity levels, ASA being the best of all. Although salinity increased the time required for emergence of seeds, but MET (Figure 2) and E_{50} (Figure 3) were significantly reduced by priming treatments, maximum reduction was recorded for ASA primed seeds followed by SA priming. Maximum emergence index was observed in control for all priming treatments and the value decreased with increase in salt stress. Emergence index was highest in ASA primed seeds followed by SA priming (Figure 4). Shoot length was also repressed by increasing salinity levels but priming treatments reduced the salt toxicity. Maximum shoot length was observed for SA priming at 3 dS m^{-1}, which was at par with ASA priming at the same salinity level (Figure 5). It is also clear from the results that seedlings from primed seeds show more shoot length at 3 dS m^{-1} and 6 dS m^{-1} than 0 dS m^{-1}. Seedlings from seeds primed with both ASA and SA had statistically similar root length (Figure 6). Root length was higher at 3 dS m^{-1} followed by 0, 6 and 9 dS m^{-1}.

LSD value = 5.487 (P<0.05)

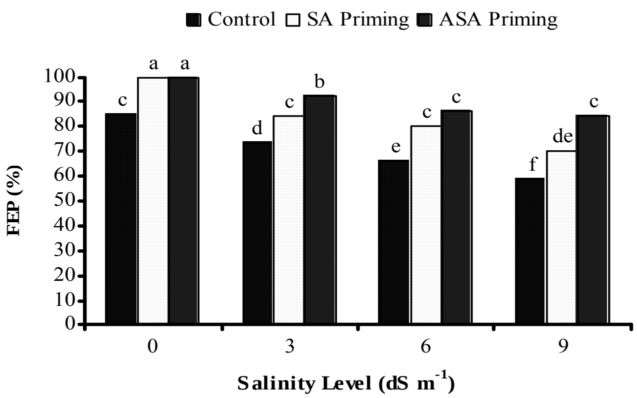

Figure 1. Effect of hormonal priming on final emergence (%) of hot pepper under saline conditions

LSD value = 4.556 (P<0.05)

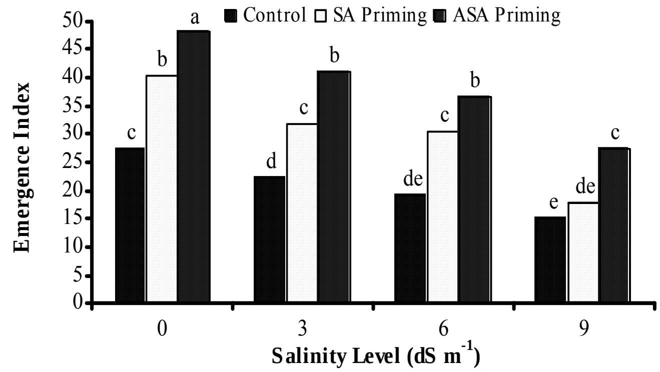

Figure 4. Effect of hormonal priming on emergence index of hot pepper under saline conditions

LSD value = 0.2356 (P<0.05)

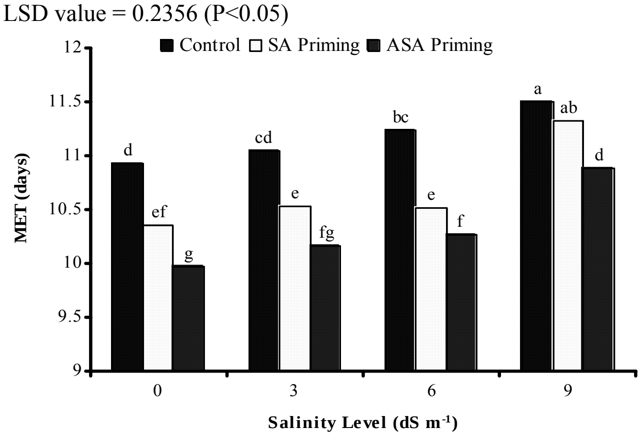

Figure 2. Effect of hormonal priming on mean emergence time of hot pepper under saline conditions

LSD value = 0.2721 (P<0.05)

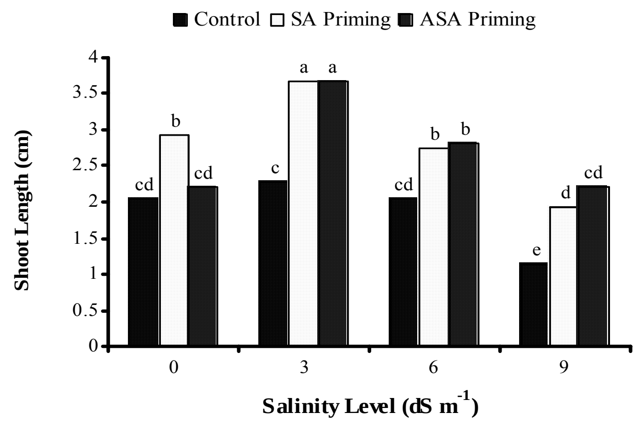

Figure 5. Effect of hormonal priming on shoot length of seedlings of hot pepper under saline conditions

LSD value = 0.9160 (P<0.05)

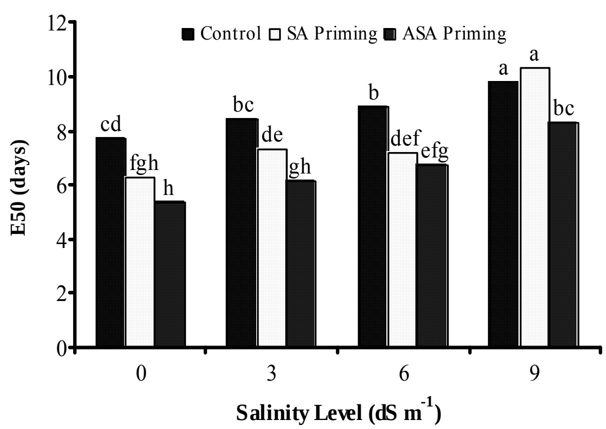

Figure 3. Effect of hormonal priming on time to 50% emergence of hot pepper under saline conditions

LSD value = 0.7438 (P<0.05)

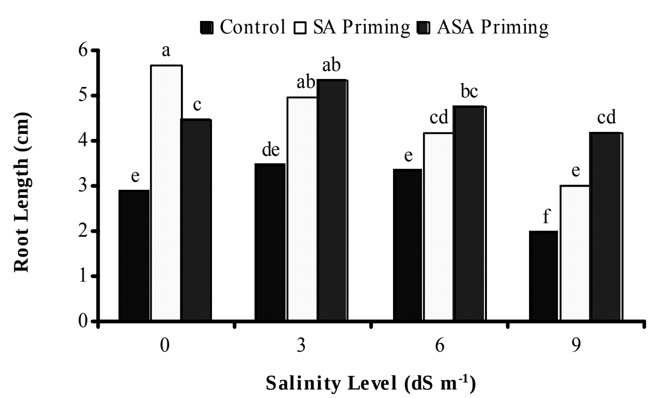

Figure 6. Effect of hormonal priming on root length of seedlings of hot pepper under saline conditions

Seedling fresh weight also increased significantly with priming treatments at different salinity levels (Figure 7). Although, priming with SA showed more seedling fresh weight but it was found statistically at par with ASA primed seeds. Seedling fresh weight was more at 3 dS m^{-1} than 0 dS m^{-1} followed by 6 and 9 dS m^{-1}. All the seedlings from different priming treatments had statistically similar dry weight but results were significant for different salinity levels (Figure 8). Interaction of both factors was also found significant. As per previous results, seedlings from primed seeds show more seedling dry weight at 3 dS m^{-1} when compared with 0 dS m^{-1} instead of high level of salinity expressing more salt tolerance. The results of vigour index proved to be highly significant (*P*<0.05) for all the priming treatments and salinity levels interaction. This is in accordance with the previous results of the experiment. Highest mean vigour index was recorded in seeds primed with ASA followed by SA as compared to control (Figure 9).

LSD value = 4.213 (P<0.05)

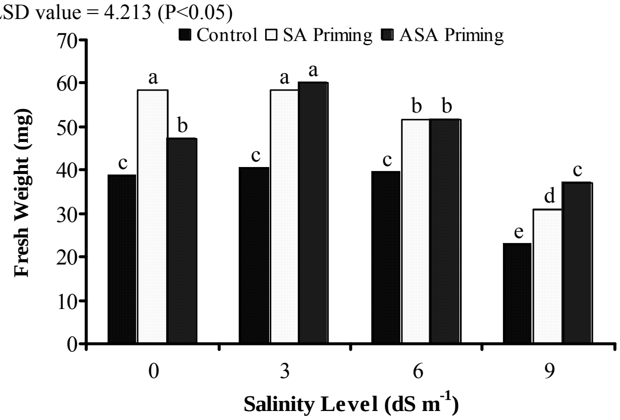

Figure 7. Effect of hormonal priming on fresh weight of seedlings of hot pepper under saline conditions

LSD value = 0.2078 (P<0.05)

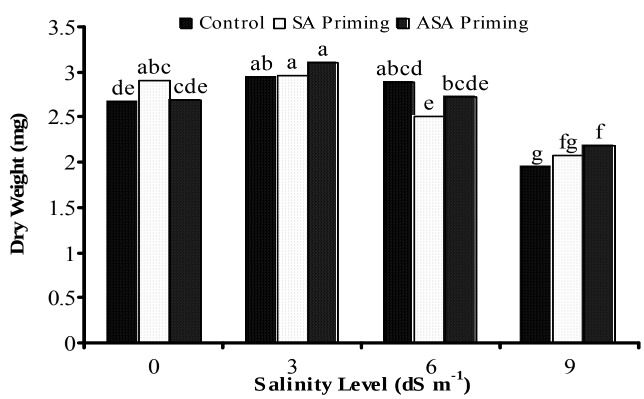

Figure 8. Effect of hormonal priming on dry weight of seedlings of hot pepper under saline conditions

LSD value = 81.39 (P<0.05)

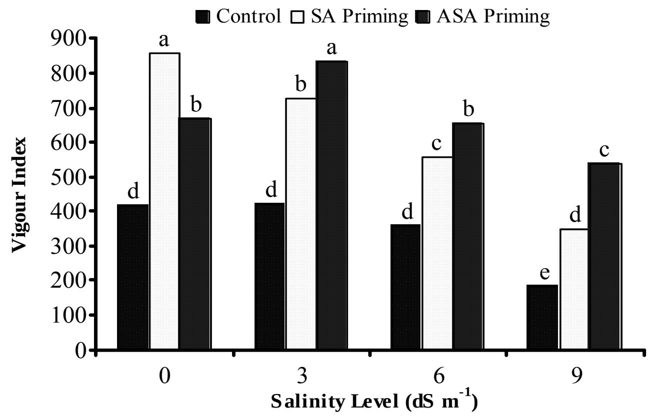

Figure 9. Effect of hormonal priming on vigour index of hot pepper under saline conditions

Discussion

Pre-treatment of seeds with different type of hormones and plant growth regulators is much effective in alleviating stress effects of salinity on the plants at different stages especially at early stage and it has been shown to improve crop germination as reported earlier under salt stress (Ashraf and Foolad, 2005; Ashraf *et al.*, 2008).

In case of FEP, priming with ASA proved to be the best treatment in terms of alleviating the adverse effects of salinity on emergence of the seeds. Maximum number of emerged seedlings will provide better early stand and also improved later growth at different stages. Under the influence of salt stress the osmotic potential ($\psi\pi$) greatly decreased and both ASA and SA pre-treatments moderated it (Szepesi *et al.*, 2005). Afzal *et al.* (2005) also found the same results under salinity stress in case of hormonal priming of wheat seeds which provide significancy to our results. Similarly, the increase in emergence percentage in seeds primed with salicylic acid may be due to enhanced oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis under saline conditions (Karthiresan *et al.*, 1984).

Earlier and synchronized emergence was observed in primed seeds compared with that of unprimed which is represented by MET and E_{50}. It was found that ASA presented the best EI at all the salinity levels competing with adverse effects of salinity which decrease the emergence of the seeds. Our results support the findings of Rivas *et al.* (1984) who observed earlier germination of primed Jalapeno pepper seeds. Increased emergence rate due to seed priming may be due to increased rate of cell division in the root tips of seedlings from primed seeds as reported in tomato (Farooq *et al.*, 2005).

Seedling growth was adversly affected under saline conditions, which is in agreement with Cicek and Cakirlar (2002) who reported that salinity reduced shoot length, fresh and dry weight of maize seedlings. Increased shoot and root length may be due to early emergence induced by priming treatment as compared to un-primed seeds. Stofella *et al.* (1992) presented the same results by observing that priming of the pepper seeds significantly improved root length. These results are also in confirmation with Tari *et al.* (2002) showing that salt tolerance is induced in seedlings raised from seeds primed with salicylic acid. Stress tolerance due to pre-treatment of seeds suggests that these molecules trigger the expression of the potential to tolerate stress rather than having any direct effect as a protectant (Senaratna *et al.*, 2000).

Adverse effects of salinity on seedling growth could also be measured in relation to seedlings fresh and dry weight. Although results were significant for improving the fresh weight of the seedlings but there was a minute difference between the fresh weight of seedling produced by primed and un-primed seeds. Khodary (2004) also found the same results that SA increases the fresh and dry weight of shoot and roots of stressed maize plants. Results are also in accordance with the findings of Afzal *et al.* (2005) for wheat seedlings primed with SA under saline conditions.

It is well established that a vigourous seed can produce a better seedling under stress conditions than the non-vigorous one. All the priming treatments showed improved VI as compared to non-primed seeds which was due to increased shoot and root length of seedlings from primed seeds and so much more vigorous than from un-primed seeds. Our results for improved vigour with SA and ASA priming are in accordance with the Afzal *et al.* (2005). Farooq *et al.* (2005) also suggested that priming treatments improves the vigour of the seeds. Increased vigour in primed seeds might be due to decreased catalase and peroxidase levels as recorded in pea seedlings treated with SA (Srivastava and Dwivedi, 1998). These results are also supported by the enhanced tolerance of tomato and bean seedlings sprayed with SA and ASA to heat, chilling and drought stress (Senaratna *et al.*, 2000). Salinity tolerance in seeds primed with SA might be the result of augmented activities of proteinase in endosperm and the contents of soluble sugar, protein and free amino acids under stress conditions (Zhang *et al.*, 1999).

Our results suggested that ASA priming alleviate the adverse effects of salinity on the seed and seedlings of the hot pepper followed by SA priming over the non-priming treatment. There is a need to have comprehensive studies on plant water relations after SA and ASA treatments to understand the measures which lead to stress tolerance.

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